

# Recovery of Bacteriophage from Contaminated Chilled and Frozen Samples of Edible West Coast Crabs

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Edible West Coast crabs (*Cancer magister* and *C. antennarius*) were contaminated with bacteriophage and then held in a chilled or frozen state. Results indicated a significant survival of virus regardless of storage conditions.

It has been demonstrated that viruses and other pathogenic microorganisms, when present in food products, can withstand freezing or chilling for a considerable length of time (1, 2, 3, 7, 8, 9, 11, 12, 13). It has recently been shown that edible crabs can also become contaminated with viruses (4, 5). However, it is not known to what extent viruses survive in these animals when they are kept in a chilled or frozen state. Therefore, a preliminary investigation into this problem has been initiated in our laboratory.

Two separate series of experiments were conducted. In the first study, edible West Coast crabs *Cancer magister* and *C. antennarius*, which have a carapace width of 13 to 16 cm, were exposed to coliphage T4 ( $5.0 \times 10^4$  plaque-forming units [PFU]/ml of seawater) for 24 h. These viruses were chosen as models because the bacteriophage have been used by other workers to study viral uptake and persistence in shellfish. (6) Furthermore, Kott et al. (10) have shown the coliphage to be as resistant to the marine environment as are enteroviruses. Therefore, it was felt that coliphage T4 would be a suitable model for the present studies.

After contamination, the crabs were divided into two equal lots, dipped in a 1% hypochlorite solution to inactivate any viruses adhering to the carapace surfaces, and then washed in distilled water. One lot was sealed in polymylar pouches, six crabs per pouch, and placed in a refrigerator set at 8 C. The second lot was processed by being boiled in water containing 0.5 g of NaCl per liter for 20 min at 100 C. This lot was then sealed in polymylar pouches, six crabs per pouch, and refrigerated. The crabs were assayed for virus content at 0, 24, 48, 72, 96, and 120 h. Samples were readied for assay

by preparing 10% (wt/vol) homogenates of tissue (crab muscle, digestive gland, and blood), with nutrient broth as a diluent. All homogenates were blended for 2 min at 6,500 rpm in a Waring blender. After clarification, serial decimal dilutions were prepared in sterile, isotonic saline, and the samples were assayed for virus by being plaqued in nutrient agar (pH 7.6; 0.0025%  $\text{CaCl}_2$ ) containing approximately  $10^8$  host cells per ml. The host cells, *Escherichia coli* B, were propagated in 250-ml flasks of nutrient broth at 37 C. The plates were incubated at 37 C for 12 h.

In the second study, the crabs were allowed to be contaminated for 24 h in seawater containing  $4.6 \times 10^4$  phage PFU per ml. Samples were assayed for viral content and found to contain  $3.6 \times 10^4$  phage PFU per g. On a per-unit weight basis, this represented a 78% uptake of the virus by the crabs. This uptake was significantly higher than that observed in a study of enterovirus uptake by shore crabs (4). In this previous study, viral uptake at 24 h was found to be only 32%. However, this difference in accumulation probably reflects the size difference existing between the species of test animals.

The remaining animals were sanitized, washed in distilled water, and divided into two equal lots. One lot was sealed in polymylar pouches, four crabs per pouch, then frozen and stored at -20 C. The second lot was processed by being boiled for 20 min, sealed in polymylar pouches, and then frozen and stored as above. Samples to be tested were allowed to thaw to room temperature before being assayed. Assays were conducted at 0-, 1-, 20-, and 30-day intervals.

In all studies, the zero-hour samples represented the titer of viruses existing in the crabs after either contamination or processing.

The results of the chilling experiments using contaminated unprocessed and processed (boiled) crabs are shown in Fig. 1. A gradual decrease in virus titer was observed in all samples during the entire test period. However, after 120 h  $7.0 \times 10^4$  virus PFU per g were still recovered from the unprocessed crabs, and 45 virus PFU per g were recovered from the processed samples. On a per-unit weight basis, these represent approximately 29 and 40% of the virus contained per gram of respective tissue sample. The studies were discontinued after 120 h because of the rapid decomposition of the unprocessed crabs.

The results of the freezing studies are presented in Table 1. There was a decrease in virus titer during the entire period of this experiment. However, the decrease was gradual; after 20 days of storage,  $1.5 \times 10^4$  virus PFU per g from the frozen unprocessed crabs and  $1.4 \times 10^2$  virus PFU per g from the processed samples were recovered. This represents survival or recovery rates of approximately 42 and 55%, respectively. At the end of the study (30 days),  $1.2 \times 10^4$  virus PFU per g were still found to be present in the unprocessed samples, and 45 PFU per g were recovered from the processed samples.

The results reported are preliminary observations. Results obtained by other workers have

TABLE 1. Recovery of coliphage T4 from contaminated samples of unprocessed and processed edible crabs stored for 30 days at  $-20^\circ\text{C}$

Sample	Storage time (days)	No. of virus in crab tissue (PFU/g <sup>a</sup> )	Survival (%)
Unprocessed crab	0	$3.6 \times 10^4$	100
	1	$3.6 \times 10^4$	100
	20	$1.5 \times 10^4$	42
	30	$1.2 \times 10^4$	35
Processed crab	0	$2.6 \times 10^2$	100
	1	$2.6 \times 10^2$	100
	20	$1.4 \times 10^2$	55
	30	45	17

<sup>a</sup> PFU, plaque forming unit.

indicated a long-term persistence of human enteroviruses in chilled and frozen foods. Lynt (12) observed that type 1 poliovirus and types B1 and B6 coxsackievirus would survive for 1 month in chilled foods and up to 5 months in frozen products. These findings were confirmed by Heidelbaugh and Giron (8) and substantiated by DiGirolamo et al. (3). However, our findings suggest that the rate of virus inactivation in crabs may be more rapid than that which occurs in other food products. Possibly this increased rate of inactivation was due in part to the thawing process and in part to autolytic reactions in the crab, either of which may have had an effect on the virus used.

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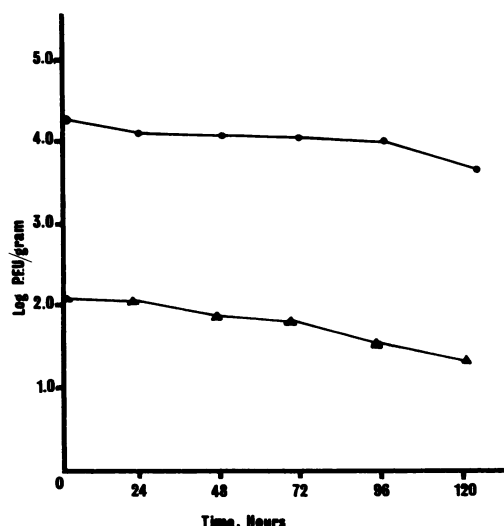


FIG. 1. Effect of storage at  $8^\circ\text{C}$  on recovery of added *Escherichia coli* B viruses ( $5.0 \times 10^4$  plaque-forming units/ml of seawater) from unprocessed and processed (20 min at  $100^\circ\text{C}$ ) crabs. Chilled, unprocessed (●); chilled, processed (▲).

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